COMPARATIVE METABOLIC EFFECTS OF FRUCTOSE AND GLUCOSE IN HUMAN FIBROBLAST CULTURES

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SUMMARY

The comparative metabolic effects of fructose and glucose were determined in human fibroblast cultures. Cells were grown in four different media containing 5.5 and 27.5 mM of glucose and fructose, respectively. For these two hexoses, we compared their uptake, consumption, and conversion into \(^1\)\(^4\)CO\(_2\) and \(^1\)\(^4\)C-lipids.

D-Fructose was taken up in fibroblasts by an unsaturable process and its consumption was much smaller than that of D-glucose. Whatever the experimental procedure, the glycogen content of cells grown in fructose media was significantly lower than that of those grown in glucose media. Labeling of fructose and glucose with \(^1\)\(^4\)C showed that more carbon from fructose than from glucose was incorporated into \(^1\)\(^4\)CO\(_2\) and glycerolipids. The relative distribution of \(^1\)\(^4\)C in the different lipid fractions was similar for both hexoses.

These results indicated that the pathways of intermediary metabolism in fibroblast cultures were influenced by the nature of the carbohydrate present in the culture medium and that fructose was a better lipogenic substrate than glucose in human fibroblast cultures.

Key words: fructose; glucose; human fibroblast cultures; lipids.

INTRODUCTION

The role of fructose as a lipid precursor has been extensively explored in vivo in connection with the metabolic disorders induced by the excessive proportion of fructose in human diet. Most of these investigations demonstrated that fructose was a good lipogenic substrate and that it stimulated lipogenic enzyme activities (28). Only a few studies were carried out in cell cultures on the effect of fructose on cellular metabolism (12,13,23). Induction of lipogenesis by fructose has been shown in primary cultures of rat hepatocytes (25,27). To our knowledge there are no reports concerning such induction in human diploid fibroblasts. Recent studies in this laboratory showed that replacement of glucose by fructose in the medium of human fibroblast cultures stimulated the DNA synthesis related with a decrease of ATP levels (6) and induced changes in cellular metabolism such as increased pyruvate dehydrogenase activity (32). Furthermore, human diploid fibroblasts seem to be a suitable model system to investigate substrate-supported lipid synthesis (22). Consequently, in this work, we studied fructose as a lipogenic precursor in human fibroblast cultures. We also compared the use of fructose and glucose in these cells and specified some steps in their metabolism.

MATERIALS AND METHODS

Cell Cultures

Abdominal skin fibroblasts came from surgical biopsies from infants who required abdominal surgery. Patients were sex-matched (8 girls and 5 boys) and their ages varied from 1 mo. to 13 yr. This work was previously submitted to the local ethical committee and approved.

Fibroblast cultures from these explants were initiated as previously described (16). Cells were grown in Eagle's minimum essential medium with nonessential amino acids (MEM Eurobio no. 2111) and supplemented with 10% human serum. Serum, undialyzed, was checked for the undetectable concentration of glucose before use.

Experimental Procedures

Chemicals. All chemicals were of reagent grade and came from Boehringer Mannheim, and organic solvents were purchased from Merck, Darmstadt, Germany. D-(U-\(^1\)\(^4\)C) glucose (300 mCi/mmol) and D-(U-\(^1\)\(^4\)C) fructose (165 mCi/mmol) were obtained from CEA, Gif-sur-Yvette, France.

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**FIG. 1.** Time course of $D$-glucose (•) and $D$-fructose (▲) uptake in fibroblast cultures. Uptake studies were carried out in medium containing 1 $\mu$Ci (U-$^{14}$C) hexose/ml PBS and 5.5 mM of glucose or fructose. Each experimental point represents the mean ± SEM of nine determinations from three cell lines.

**D-Glucose and D-Fructose uptake.** Time-course determinations (1 to 30 min) were performed on the fibroblasts grown in standard medium on rectangular cover slips (Thermanox, 24 × 30 mm, Lux Scientific) deposited on an 8-well multiplate ($1 \times 10^4$ cells/well).

At cell subconfluency the medium was removed and replaced by the same medium free of hexose. This procedure was selected because when glucose was removed from the medium the hexose uptake was increased (15,17). Twenty-four hours later the cells were washed with phosphate buffered saline (PBS), and at zero time the labeled sugar, $D$-($U$-$^{14}$C) glucose or $D$-($U$-$^{14}$C) fructose, was incorporated with 5.5 mM of glucose or fructose ($1 \mu$Ci/ml PBS). After incubation cells were prepared for kinetic study according to the procedure of Vessal et al. (27). Briefly, the cover slip with attached cells was first immersed in the labeled medium for the specific time period and then washed for 30 s in each of eight beakers in succession containing 50 ml cold 0.9% NaCl. The washed cover slip was transferred in 0.1 N NaOH. Cells were removed and sonicated. An aliquot was used for measurement of radioactivity by liquid scintillation spectrometry, and another aliquot was used for the determination of protein (18). The time courses for the uptake of the two sugars were determined concomitantly in each subculture. Kinetic experiments were conducted at fructose concentrations between 0.050 and 100 mM.

**Glucose, fructose, and lactate determinations.** The cells, plated at $4 \times 6 \times 10^4$ cells/flask (Falcon Plastics, 25 cm²), were grown in replicate flasks. Each flask contained 4 ml of one of four media: $D$-glucose, 5.5 mM (G 5.5), or 27.5 mM (G 27.5) and $D$-fructose, 5.5 mM (F 5.5) or 27.5 mM (F 27.5). All four media were concomitantly collected after 3, 4, or 6 days of culture. Glucose, fructose, and lactate were measured by standard enzymatic methods (Boehringer Kit) in nutrient medium after deproteinization with perchloric acid.

**CO$_2$ production.** $^{14}$CO$_2$ production from $D$-($U$-$^{14}$C) fructose or $D$-($U$-$^{14}$C) glucose was measured as described previously (31). Briefly, on the day before the assay cells were plated into the wells of a microtiter plate (Falcon Plastics, type 3040) at a density of about $2 \times 10^4$ cells/200 $\mu$l of hexose-free medium. At zero time the medium was removed, and the cells were washed with Krebs-Ringer phosphate buffer. About 0.5 $\mu$Ci labeled sugar was added to each well, so that the final concentration was 5.5 or 27.5 mM. Wells were covered with 10-mm glass fiber discs (Whatman, type GF1D) soaked with 3.5 N NaOH, and cells were incubated for either 1 or 3 h at 37$^\circ$C. Each disc was transferred in a liquid scintillator and counted.

**Activity of fructokinase** (ketohexokinase - EC 2.7.1.3). This activity was measured as described by Weiser and Quill (30).

**Glycogen content.** Intracellular glycogen was determined by an enzymatic fluorometric micro method (19). Glycogen content was expressed as nanomoles of glucose per milligram of total protein. To specify the glycogen-sis capacity of fibroblasts two experimental procedures were carried out. In procedure I, 1.0 to 1.5 $\times 10^4$ fibroblasts were plated/flask (Falcon Plastics 75 cm²) and were maintained for 72 h in the four mediums (G 5.5, G 27.5, F 5.5, F 27.5). Cells were detached by gentle scraping after addition of 10 ml cold isotonic saline solution. In procedure II cells were incubated in hexose-free medium for 72 h, placed for 24 h in the appropriate medium (G 5.5, G 27.5, F 5.5, F 27.5), and detached.

**Cellular lipids.** Twenty-four hours before the experiment, at cell subconfluency, the standard medium was

**FIG. 2.** $D$-fructose uptake as a function of fructose concentrations. Cells were incubated in PBS at 37$^\circ$C for 1 min at fructose concentrations of 1 to 100 mM. Each experimental point represents the mean ± SEM of nine determinations from three cell lines.